Influence of seed location on cotton fiber development in planta and in vitro

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Abstract

A number of different levels of competition for resources have been described that influence cotton (*Gossypium hirsutum* L.) fiber quality. These include the number of bolls on a fruiting branch, and on a secondary level, the location of seeds within a locule. Cotton ovule culture was employed to assess the contribution of competition for resources to fiber quality. Elongation and secondary wall deposition were compared in plant-grown and culture-grown fibers. In plant-grown material, seeds located in the middle of a locule had the longest fibers. Fiber length distributions were similar for ovules taken from the apical, medial and basal locations in a locule and grown in ovule culture. Secondary wall deposition was greatest in fibers located on basal seeds or ovules. On a single seed or ovule, fibers in the micropylar region had thicker cell walls than in the chalazal region. Competition for resources has been eliminated in vitro and the same pattern of secondary wall deposition occurred in the micropylar and chalazal regions of seeds and ovules. Therefore, conditions present at the time of ovule excision influenced secondary wall deposition. One condition may be fiber diameter.

1. Introduction

Cotton plants initiate some fruiting buds that never mature into harvestable bolls. Translocation of assimilates out of branches bearing bolls is low [1–4]. The lack of synchronization between carbon production and utilization by leaves and bolls necessitates that boll growth rely on imported assimilate [5]. Main stem leaves contribute significantly to the carbon requirement of the first

fruiting position on a branch but very little to other positions on the same branch. Leaves along the fruiting branch export most of their assimilate to bolls on the same fruiting branch [5]. Bolls at the first position are larger than those at the second and third positions on a fruiting branch [6]. Under conditions in which boll load was light, boll size, fiber length and fiber strength increased [7]. Competition at this branch level revealed that bolls at the third position on a branch had shorter and less mature fibers than bolls at the first position [8].

In addition to competition for assimilates among bolls on the same fruiting branch, a second level of competition occurs among seeds within a

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boll. Bolls have 3–5 locules each containing 7–10 seeds. Seeds near the apex or base of a locule have shorter fibers than seeds at the middle positions [9]. Length variation may be inherent and/or due to unequal assimilate partitioning during fiber and seed development. Another component of fiber quality, the degree of secondary wall deposition also varies with seed location in a locule. One of the difficulties in evaluating alteration of fiber characteristics lies in the fact that they have an asymmetric frequency distribution [10]. A component of this asymmetrical frequency distribution is found in a single seed since examination of individual regions on a single seed revealed the same asymmetric fiber length distributions [10]. Although each seed region has an asymmetric fiber length frequency distribution, the mean fiber length is shorter at the micropylar end of a seed than at the chalazal end [11].

Cotton ovule culture has been used to study the hormonal and nutritional requirements for fiber growth and development [12,13]. Fiber development is similar in field-grown ovules and in ovules grown in vitro [14]. Cotton fibers are derived from epidermal cells in the outer integument of the ovule. The period of maximum fiber elongation, duration of fiber elongation and the timing of the transition from primary to secondary wall synthesis occur earlier in vitro [14]. Fiber development does not depend on the presence of an embryo since unfertilized ovules produce fiber in culture [13]. In plant-grown seeds differences in fiber properties may be due to the location of the seeds in a locule. If ovules are removed from specific locations in a locule and are grown in culture, they are not subjected to the same competition for resources that may be found in plant-grown material. Experiments reported in this paper were conducted to ascertain the extent that intra locule competition for resources affects fiber properties.

2. Materials and methods

2.1. Plant material

Cotton (Gossypium hirsutum L.) cultivar Texas Marker 1 was grown under standard greenhouse conditions (minimum night temperature 20°C, maximum day temperature 35°C) with 14 h/day il-lumination in the autumn of the year 1992. The natural photoperiod was supplemented with light from 400-W sodium vapor lamps. First position flowers were tagged on the day of anthesis.

2.2. Fiber analysis of dried fiber with AFIS

The final period in boll development is boll opening (dehiscence). When bolls open, the fibers lose water and dry out. The tubular fiber cells become twisted ribbons that cannot easily be reconstituted as tubular cells. Seeds were collected from open bolls and from bolls opened manually prior to dehiscence and allowed to air dry. Bolls were collected from the second to eighth fruiting branches. Fiber was removed and analysed using the Zellweger Uster Advanced Fiber Information System (AFIS) [15]. An individual fiber is separated from a fiber sample and transported to an electro-optical sensor in which the extinction-mode (V_e) and scatter mode (V_s) signals are measured simultaneously. The computer software analyses the electrical impulses of both signals; the data from the V_e signal yields individual fiber length and diameter while data from the V_s signal yields circularity, cross sectional area, and micronafis. Cottons of known micronaire values were used to calibrate the micronafis values so that micronafis is comparable with micronaire. Micronaire is an indirect measurement in which air is forced through a sample and the resistance to air flow is proportional to linear density (weight per unit length of fiber). Micronaire readings are affected by fiber maturity, since maturity has been defined as the relative wall thickness of fibers.

Apical and basal seeds from the same locule were collected from open bolls. Fibers from the micropylar region of 16 apical seeds were removed and pooled into one replicate. The same was done with the chalazal region of the apical seeds. Fibers from the micropylar and chalazal regions of 16 basal seeds were collected in a similar manner. Three replicates were analysed. Some 4000–8000 fibers per replicate were measured by AFIS. Seeds were removed from bolls opened manually and allowed to air dry. All fibers from four apical seeds from four bolls were pooled into a single sample. All fibers from four seeds from basal locations were pooled and analysed with AFIS. This latter

set of samples was compared with fiber analysis done using a microscope.

2.3. Fiber analysis of never-dried plant-grown fiber

Some bolls were harvested 3, 4 and 5 weeks after flowering to assess seed and fiber growth; remaining bolls were harvested 6 weeks after flowering. Bolls were collected from the third to eighth fruiting branches. The boll wall was cut open and the contents of each locule were removed. Fibers were not allowed to dry out. Fiber length was determined by placing seeds into a beaker of boiling water for 2 min. This allowed the fiber to fluff out. The seeds were then placed on the convex side of a watch glass and the fiber was separated under a stream of water [16]. Fiber length was recorded as the length of fibers from the middle region of each seed. Before the seeds were placed in boiling water small fiber samples were removed from five areas on each seed. Ten bolls were examined.

2.4. Fiber wall thickness measurements of never-dried fiber

Never-dried fibers were examined with an Olympus BH2 microscope fitted with an ocular micrometer. Magnification was adjusted so that each micrometer subdivision was 2 μm. Fiber diameters ranged from 20 to 40 μm. Fiber diameter near the base of the fiber increases slightly in the period from 2 to 5 days post anthesis [17]. Secondary wall deposition proceeds from the primary cell wall toward the center of the fiber. In some fibers the entire cell lumen is composed of secondary wall. At least one-half of a fiber lumen had to be composed of secondary wall to receive a score of 1. A fiber with less than half of the lumen composed of secondary wall received a score of 0. Five areas were sampled for each seed or ovule. Twenty fibers from each area were examined for a total of 100 fibers per seed. Therefore, if a seed had 35 fibers with a score of 1, then the Wall Value was 35. Wall Value is the percentage of fibers on a seed with a score of 1.

2.5. Ovule culture

Bolls were collected 30 h post anthesis and surface sterilized in a 50% solution of commercial bleach (5.25% NaOCl) for 10 min. After rinsing in sterile distilled water, ovules were removed and floated on ovule culture medium (pH 5.0) containing 5.7 μM gibberellic acid and 5 μM indole-3-acetic acid [18]. Cultures were grown in the dark at 32°C. This medium did not permit embryo development. Embryo development is not an important contributor to fiber development in vitro since Meinert and Delmer [14] have shown that fibers on unfertilized ovules grown in a medium containing indole-3-acetic-acid and gibberellic acid were similar to plant-grown fibers in terms of composition and in terms of relative changes in composition during development. Ovules from the same locule location from one boll were placed into small Petri plates containing 10 ml of medium. Eight bolls were cultured. Medium was replaced after 2 weeks in culture and the ovules were examined after 6 weeks in culture. Fiber length was not measured in the same manner as it was for plant-grown fibers since culture-grown fibers were not as dense as fibers on seeds. Also, it was easier to measure fiber length, in the manner described below, and then immediately examine the fibers for wall thickness. In order to measure fiber length an ovule was placed on a glass slide, cut half way through and pulled apart to separate the fibers [18]. Fiber lengths for fibers in the middle of the ovules were recorded. Wall Values were determined on a per-seed basis after examining 100 fibers. For some ovules specific regions of the ovule were examined. On 5 bolls 40 fibers were measured for each region (micropylar and chalazal). A Regional Wall Value was calculated for 4 ovules from a single locule location and expressed as Regional Wall Value per boll.

3. Results

3.1. Plant-grown fiber

Micropylar and chalazal regions on a single seed. At anthesis, fibers first appear at the crest of the funiculus. Fiber initials are delayed for a few hours at the chalazal region, and for 3 or 4 days at the micropylar region [19]. Fiber initials differentiate from single epidermal cells. All ovules in a locule initiate fiber elongation on the day of anthesis. Seeds were harvested from open bolls and sorted according to locule location. The seed was divided

Table 1
Characteristics of Texas Marker 1 greenhouse-grown fiber as measured with the advanced fiber information system

Locule location	Seed region	Length (w) mm	Length (n) mm	UQL (n) mm	Diameter μm	Micronafis
Apical	M	27.6 ± 0.5	25.9 ± 0.5	30.5 ± 0.5	14.7 ± 0.5	6.4 ± 0.7
Apical	C	29.5 ± 0.5	27.7 ± 0.5	32 ± 0.2	14.6 ± 0.3	5.6 ± 0.7
Basal	M	24.9 ± 0.8	23.4 ± 0.8	27.2 ± 0.5	16.1 ± 0.4	7.8 ± 0.3
Basal	C	29.5 ± 0.5	27.7 ± 0.5	32.2 ± 0.2	13.1 ± 0.2	5.5 ± 0.1

Length (w) is the length by weight value. Length (n) is the length by number value. UQL (n) is the upper quartile mean length of fibers using the length by number value. Data are given as means of three replicates ± S.E.
Seed regions: M, micropylar region; C, chalazal region.

into micropylar, middle and chalazal regions. Fibers from the micropylar and chalazal regions were removed and analysed using AFIS (Table 1). Data obtained from this instrument are similar to the more standard HVI (High Volume Instrument) measurements used to measure fiber quality [20]. The conventional method of assessing fiber length by weight–length measurement in which fibers are sorted into common-length groups and then weighed tends to hide short fibers. If the number of fibers in each length group is determined by counting or by dividing the group weight by the fiber weight and the number fraction of each length group is plotted against length, a number–length distribution is obtained. The number–length plots tend to emphasize short fibers [21]. Both types of measurements were performed using AFIS. Additionally, the upper quartile mean length (UQL) of fibers was recorded. This gave an indication of the longest fibers in the sample. Ribbon width (width of the collapsed fiber) is described by the diameter measurement.

Micronafis is a measurement similar to micronaire. AFIS was calibrated with fibers of known micronaire. In plant-grown material apical and basal seeds from the same locule were compared. Short fibers occurred in the micropylar region of the seed and the shortest occurred in basal seeds. Plant-grown fibers in the micropylar region of basal seeds were the most mature. Fibers in the chalazal region of both apical and basal seeds were the same length and maturity. When fibers from any region of a cotton seed were removed and measured a normal frequency of fiber lengths was observed [21]. The length value from AFIS is the mean length.

3.2. Seed location in a locule

Seeds were harvested from plant-grown bolls 1 week before boll opening. Fiber length and the extent of secondary wall deposition (Wall Value) were recorded for seeds from the apical, medial, and basal regions of the locules (Figs. 1 and 2). The procedure for measuring fiber length tended
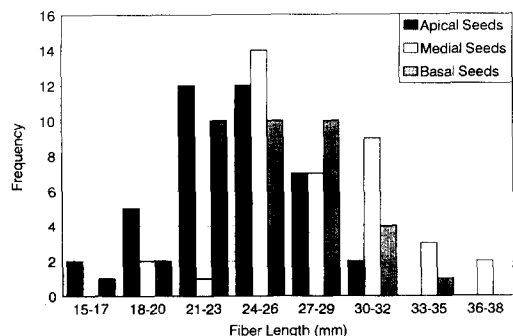

Fig. 1. Fiber length distributions for plant-grown fibers of Texas Marker 1 at 6 weeks post anthesis. Frequency equals number of seeds.
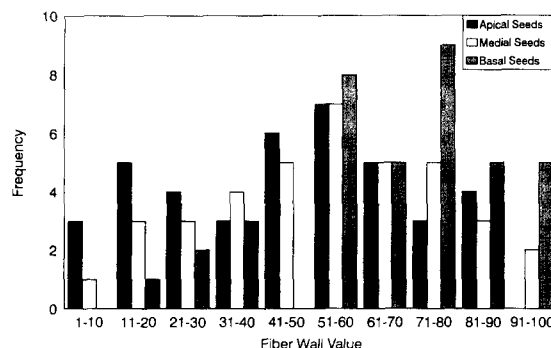

Fig. 2. Fiber wall value distributions for plant-grown fibers of Texas Marker 1 at 6 weeks post anthesis. Frequency equals number of seeds.

to overestimate fiber length since never-dried fibers can be stretched during the procedure. Fibers from the middle region of each seed were measured to give a middle value since mean fiber lengths differ at the micropylar and chalazal regions of a seed. Fiber length distributions peaked at 21–29 mm for all seed locations. More apical and basal seeds had shorter fibers than medial seeds. Fiber Wall Values had wide distributions but generally the basal seeds had higher Wall Values than apical or medial seeds. Fiber Wall Values included fiber from all regions of the seed and was thus a total seed value. A comparison of fiber Wall Values and micronafis values showed that fibers from apical and basal seeds with high Wall Values (80–90) had micronafis values of 6.3–6.5.

3.3. Culture-grown fibers

Micropylar and chalazal regions on a single ovule. Fiber growth and development in ovule culture exhibits a compressed schedule [14]. Ovules were grown in culture for 6 weeks. Forty fibers were measured in the micropylar and chalazal region of the ovules. Ovules from each locule location (apical, medial and basal) were cultured separately. The four ovules per Petri plate were from a single boll. The Regional Wall Value was based on 40 fibers per ovule and the Regional Wall Values were combined to give a per boll Regional Wall Value. Fiber from the micropylar region of ovules grown in culture showed a tendency toward higher Regional Wall Values than fiber on the chalazal region of the same ovules (Table 2). Fibers from
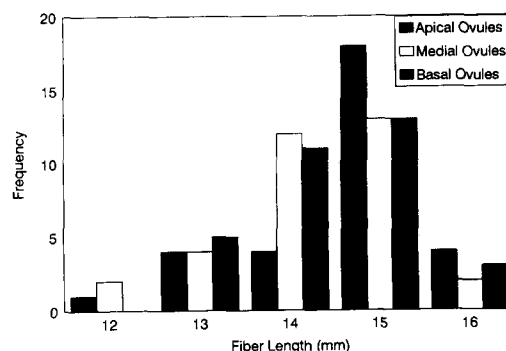

Fig. 3. Fiber length distribution for culture-grown fibers of Texas Marker 1 after 6 weeks in culture. Frequency equals number of seeds.

the micropylar region of basal ovules had higher Regional Wall Values than did those from apical ovules.

3.4. Ovule location in a locule

Fiber elongation in vitro is always less than in planta and the distribution range was more narrow than that for plant-grown fibers (Fig. 3). Fiber length distributions peaked around 14–15 mm. No differences in fiber length distributions were seen among apical, medial and basal ovules. Fiber Wall Values were low but the distribution of Wall Values for fibers grown in vitro revealed that fibers on the apical ovules had the lowest Wall Values, and hence, thin secondary walls while fibers on basal ovules had thicker secondary walls (Fig. 4).

Table 2
Secondary wall deposition in culture-grown fiber

Locule location	Ovule region	Regional wall value per boll
Apical	Micropylar	6 ± 1
	Chalazal	2 ± 2
Medial	Micropylar	10 ± 6
	Chalazal	6 ± 3
Basal	Micropylar	15 ± 5
	Chalazal	6 ± 6

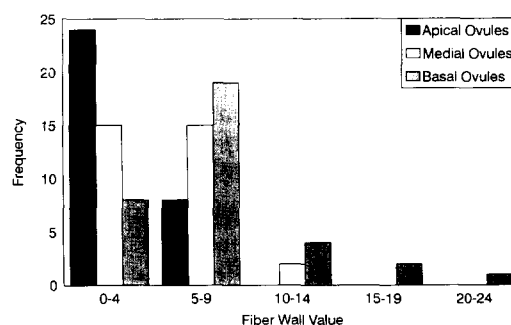Data are given as means of five replicate bolls ± S.E.

Fig. 4. Fiber wall value distributions for culture-grown fibers of Texas Marker 1 after 6 weeks in culture. Frequency equals number of seeds.

4. Discussion

4.1. Micropylar and chalazal regions on a single seed

The disparity in fiber lengths in the micropylar and chalazal regions of the seed could be explained by the duration of the elongation stage of fiber development. Since fibers in the chalazal region begin elongation before those in the micropylar region, one would expect both plant-grown and culture-grown fibers in the chalazal region to be longer than fibers in the micropylar region. The differences in fiber lengths in the micropylar regions of apical and basal seeds may be related to the rates of cell elongation in the micropylar regions of seeds.

Perhaps each fiber cell receives a certain allotment of assimilates during the period of secondary wall deposition. The allotment could be similar for each fiber cell on a seed, but different for seeds in different locule locations. Fibers in the micropylar regions of basal seeds have larger diameters than fibers in the chalazal regions (Table 1). Fiber perimeter can be calculated from AFIS measurements. Fiber perimeters in the micropylar and chalazal regions of apical seeds were 52.3 ± 0.7 μm and 53.2 ± 0.8 μm, respectively. Fiber perimeters of basal seeds were 53.1 ± 0.3 μm and 50.3 ± 0.5 μm, respectively. Secondary wall deposition starts prior to the cessation of elongation but elongation ceases for all regions on a seed about the same day post anthesis [22]. Meinert and Delmer [14] found an abrupt increase in the rate of cellulose synthesis at about 16 days post anthesis (DPA). Non-fiber epidermal cells also start secondary wall formation at 16–19 DPA [17,23]. If the rate of cellulose deposition is constant per unit area of plasma membrane, the fiber with the larger volume would have more surface area for cellulose synthesis and manifest a higher micronafis. At micronaire values greater than 2.98–4.97, cotton fiber diameter distributions, as measured by AFIS, correlated with micronaire. AFIS diameter frequency distribution curves shifted as the micronaire increased [24]. If fiber diameter is an important factor in fiber quality and the diameter of the fiber is determined by the size of the epidermal cell around the time of anthesis, then fiber from ovules placed in culture 30 h post

anthesis should show similarities to plant-grown fiber. Regional Wall Values for fibers from the micropylar and chalazal regions of basal ovules showed the same trends as micronafis for fibers from basal seeds. Conversely, fiber cells in the micropylar region may have higher rates of cellulose deposition than other regions of ovules or seeds eventually leading to differences in Regional Wall Values and micronafis values.

4.2. Seed location in a locule

The second level of competition for resources occurs between seeds in a boll. Embryo development was the slowest in seeds located at the base of a locule [25]. Measurements of fiber length were made from the middle region of both seeds and ovules (Figs. 1 and 3). The longest fibers were found on medial seeds (Fig. 1). Photosynthate delivered during fiber elongation may be allocated preferentially to seeds in the middle of the locule (medial seeds). In the absence of any preferential allocation of phytosynthate (i.e., ovule culture) fiber length distribution bar graphs were similar for fibers from ovules excised from apical, medial and basal locule locations. Since fiber samples were taken from the entire seed or ovule, the Wall Values recorded represented the degree of secondary wall deposition for the whole seed or ovule. The frequency distribution showed that fibers on basal seeds and ovules had the thickest cell walls (Figs. 2 and 4). Some factors related to locule location present at the time of ovule excision predisposed the culture-grown fibers to deposit secondary wall in a manner similar to plant-grown fiber.

The growth and development of culture-grown fibers revealed that, in the absence of competition for assimilates, differences in fiber length seen in plant-grown fibers were eliminated. Reduced competition for assimilates in plants having a low boll load permitted greater fiber elongation [7]. Therefore, fiber elongation in planta was influenced by resource allocation within a locule. An underlying factor in the deposition of secondary wall may be the diameter of fiber cells at anthesis. The same trends seen in cell wall thickness in plant-grown fiber were found in culture-grown fiber.

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